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# Pharmacokinetic-Pharmacodynamic Relationships of Immunoglobulin Therapy for Envenomation

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## Abstract

Parenteral administration of horse- and sheep-derived antivenoms constitutes the cornerstone in the therapy of envenomations induced by animal bites and stings. Depending on the type of neutralising molecule, antivenoms are made of: (i) whole IgG molecules (150 kDa), (ii) $F(ab')_2$ immunoglobulin fragments (100 kDa) or (iii) Fab immunoglobulin fragments (50 kDa). Because of their variable molecular mass, these three types of antivenoms have different pharmacokinetic profiles. Fab fragments have the largest volume of distribution and readily reach

extravascular compartments. They are catabolised mainly by the kidney, having a more rapid clearance than $F(ab')_2$ fragments and IgG. On the other hand, IgG molecules have a lower volume of distribution and a longer elimination half-life, showing the highest cycling through the interstitial spaces in the body. IgG elimination occurs mainly by extrarenal mechanisms. $F(ab')_2$ fragments display a pharmacokinetic profile intermediate between those of Fab fragments and IgG molecules.

Such diverse pharmacokinetic properties have implications for the pharmacodynamics of these immunobiologicals, since a pronounced mismatch has been described between the pharmacokinetics of venoms and antivenoms. Some venoms, such as those of scorpions and elapid snakes, are rich in low-molecular-mass neurotoxins of high diffusibility and large volume of distribution that reach their tissue targets rapidly after injection. In contrast, venoms rich in high-molecular-mass toxins, such as those of viperid snakes, have a pharmacokinetic profile characterised by a rapid initial absorption followed by a slow absorption process from the site of venom injection. Such delayed absorption has been linked with recurrence of envenomation when antibody levels in blood decrease.

This heterogeneity in pharmacokinetics and mechanism of action of venom components requires a detailed analysis of each venom-antivenom system in order to determine the most appropriate type of neutralising molecule for each particular venom. Besides having a high affinity for toxicologically relevant venom components, an ideal antivenom should possess a volume of distribution as similar as possible to that of the toxins being neutralised. Moreover, high levels of neutralising antibodies should remain in blood for a relatively prolonged time to assure neutralisation of toxins reaching the bloodstream later in the course of envenomation, and to promote redistribution of toxins from extravascular compartments to blood. Additional studies are required on different venoms and antivenoms in order to further understand the pharmacokinetic-pharmacodynamic relationships of antibodies and their fragments and to optimise the immunotherapy of envenomations.

Envenomations caused by animal bites and stings represent a relevant public health problem around the world.[1-3] In the case of snakebites, it has been estimated that more than 5 million cases occur per year,[3] with estimates of fatalities ranging from 50 000–100 000.[1-3] Spider bites and scorpion stings also represent an important health hazard in many regions.[4,5] Envenomations are also inflicted by other groups of animals such as fish, molluscs, coelenterates and insects.[6] In addition to lethality, some envenomations are associated with permanent tissue damage and disability, as in the case of necrotic arachnidism and bites by viperid snakes,[1,4] thereby having a serious social and economic impact, particularly for developing countries.

Animal venoms comprise a bewildering variety of toxins, most of them being proteins and polypeptides, which exert multiple deleterious effects. Some venoms, such as those of scorpions and snakes of the families Elapidae and Hydrophiidae, contain potent

neurotoxins of low molecular mass (LMM) [<9 kDa].[7,8] Among many pharmacological effects, some neurotoxins act on voltage-dependent sodium, potassium and chloride channels, i.e. scorpion neurotoxins,[8] whereas others bind to the cholinergic receptor at the motor endplate, blocking neuromuscular transmission, i.e. elapid and hydrophid snake α-neurotoxins.[7] Toxic phospholipases $A_2$ (monomeric molecular mass ~14 kDa) are present in many snake and insect venoms.[9] They display a wide variety of toxic effects such as presynaptic neurotoxicity, myotoxicity, inhibition of platelet aggregation and pro-inflammatory activity.[9,10] In addition, many animal venoms contain high molecular mass (HMM) proteins, such as the neurotoxin of the black widow spider *Latrodectus* sp.,[11] as well as metalloproteinases and serine proteinases, abundant in viperid snake venoms, which are responsible for local tissue damage and systemic haemodynamic and coagulation disturbances.[12,13] A plethora of proteins of varying structures, displaying a wide variety of pharmacological effects, have been characterised from animal venoms. Such an extensive structural spectrum of toxins, together with the different strategies employed in the injection of these secretions and the highly variable and complex pathophysiology of envenomations, make their treatment a challenging task.

The parenteral administration of antibodies or antibody fragments has become the mainstay in the therapy of envenomations induced by animal bites and stings[14] since the development of the first antivenoms in the late 19th century.[15] Such antibody preparations, known as antivenoms, are produced in many laboratories worldwide from the plasma of animals, mainly horses and sheep, immunised with venoms.[16-19] Thus, antivenoms are made of polyclonal antibodies of heterologous origin. Attempts have been made to produce antivenoms from egg yolk antibodies,[20,21] but there are no published reports on their use in treating human envenomations. Despite some successful experimental attempts to produce neutralising monoclonal antibodies against snake and arthropod venom toxins,[22-25] there have been no clinical trials involving this type of antibody in the treatment of envenomations. Some antivenoms are 'monovalent', prepared from the plasma of animals immunised with the venom of a single species, whereas others are 'polyvalent', prepared through immunisation with a mixture of venoms from various species.[17,18]

The ways antivenoms are manufactured vary between laboratories. Based on the molecular mass of the neutralising molecule, three main types of products are currently available:

(i) whole IgG antivenoms, in which the intact immunoglobulin molecules (150 kDa) are purified either by ammonium sulphate[26] or caprylic acid[27] precipitation techniques;

(ii) $F(ab')_2$ antivenoms, obtained by pepsin digestion of whole IgG molecules, followed by ammonium sulphate precipitation of the $F(ab')_2$ antibody fragments (100 kDa);[16,28]

(iii) Fab antivenoms, prepared by papain digestion of immunoglobulins, yielding monovalent Fab fragments (50 kDa).[29]

Some manufacturers include additional purification steps, aimed at obtaining products of improved purity and safety, such as ion-exchange and affinity chromatographies[30-32] and pasteurisation.[30]

There is currently a vivid discussion on the optimal type of antivenom in terms of safety and efficacy. Antivenoms must be safe, and active efforts are being made to reduce the incidence of adverse reactions associated with antivenom therapy. Such safety seems to depend on the purity of the preparation, as well as on the reduction in the total amount of exogenous protein administered in a treatment, and in the elimination of anticomplement activity from these products.[33,34] On the other hand, antivenom efficacy depends on both the specificity and affinity spectra of antibodies to venom components

and their ability to reach such components *in vivo*. Since different antivenoms are made of molecules of variable molecular masses, they have different pharmacokinetic profiles, with evident potential pharmacodynamic implications. Owing to the relevance of these products in the therapy of animal-induced envenomations, the present review focuses on the pharmacokinetic-pharmacodynamic relationships of antivenoms.

## 1. Pharmacokinetics

### 1.1 Methodological Aspects

Experimental studies on the pharmacokinetics of antivenoms have been performed mainly in rabbits, although some investigations have used rats and mice. In some cases the antibodies have been radiolabelled with $^{125}I$,[35-37] whereas other studies have relied on immunoassays to quantify antibody concentration in blood or in tissues. The most widely used immunoassay in pharmacokinetic studies is the enzyme-linked immunosorbent assay (ELISA),[38] although immunoradiometric assay (IRMA) has also been utilised.[39] Most investigators use the intravenous route of administration, although the intramuscular and subcutaneous routes have been also employed in order to simulate the way antivenom therapy is sometimes performed in the field. Clinical pharmacokinetic analyses have mainly relied on ELISA to quantify antibody levels in serum.[40-42] A radioimmunoassay was developed for quantification of sheep Fab fragments.[43]

### 1.2 Clinical Studies

A number of studies have assessed the main pharmacokinetic parameters of antivenoms made of either whole IgG, F(ab')$_2$ or Fab (see table I). Antivenoms were administered intravenously, either by bolus injection over a 10-minute period or diluted in isotonic saline solution and infused over 30–60 minutes.[40,42,44,45] Despite quantitative variations between the studies, a general trend was observed, since Fab and F(ab')$_2$ antivenoms have a larger volume of distribution than IgG antivenoms (table I). Such large volume of distribution of Fab fragments is compatible with observations performed with anti-digoxin ovine Fab preparations.[43,46] Upon intravenous administration of IgG and F(ab')$_2$ antivenoms, a biphasic decline in serum IgG or F(ab')$_2$ concentration was observed. After a rapid early decline (distribution phase), there was a slower first-order decline (terminal elimination phase).[40] A relatively rapid decline from the central compartment, i.e. plasma, occurred.[40] Very little IgG and F(ab')$_2$ fragments were detected in the urine of these patients, suggesting that renal filtration is not a predominant route of elimination of these heterologous antibodies.[40] In turn, Fab fragments had a short

**Table I.** Pharmacokinetic parameters of antivenoms after intravenous administration in clinical studies. Values are mean ± SD or mean (range)

| Study | Type of antivenom | Number of patients | Vd (mL/kg) | $t_{½\alpha}$ (h) | $t_{½\beta}$ (h) | CL (mL/h/kg) |
|---|---|---|---|---|---|---|
| Ho et al.[40] | Horse unrefined | 7 | 90 (44–102) | 0.46 (0.14–5.2) | 82 (47–116) | 0.63 (0.38–1.48) |
| Ho et al.[40] | Goat IgG | 6 | 92.5 (58–118) | 1.96 (0.22–5.62) | 45.5 (34–72) | 1.3 (1.1–1.6) |
| Ho et al.[40] | Horse F(ab')$_2$ | 5 | 233 (177–387) | 0.3 (0.2–2.31) | 96 (79–132) | 1.67 (0.91–2.54) |
| Than et al.[48] | Horse F(ab')$_2$ | 6 | | | 36 (26–50) | |
| Meyer et al.[44] | Horse F(ab')$_2$ | 6 | | | 18 ± 4.1 | |
| Meyer et al.[44] | Sheep Fab | 9 | | | 4.3 ± 1.3 | |
| Ariaratnam et al.[42] | Sheep Fab | 11 | | | 28.1 ± 18.7 | |
| Seifert and Boyer[49] | Sheep Fab | 4 | 110 | 2.7 | 18 | |

**CL** = clearance; $t_{½\alpha}$ = distribution half-life; $t_{½\beta}$ = elimination half-life; **Vd** = volume of distribution.

**Table II.** Pharmacokinetic parameters of antivenoms after intravenous administration in experimental studies. Values are mean ± SD or mean ± SEM

| Study | Type of antivenom | Animal model | Vd (mL/kg) | $t_{½\alpha}$ (h) | $t_{½\beta}$ (h) | CL (mL/h/kg) |
|---|---|---|---|---|---|---|
| Ismail & Abd-Elsalam[35] | Horse IgG | Rabbit | | Rapid initial phase 2.12 ± 0.38; intermediate phase 5.22 ± 0.98[a] | 46.4 ± 4.8 | 0.36 ± 0.06 |
| Ismail et al.[36] | Horse IgG | Rabbit | 162 ± 1.9 | Rapid initial phase 0.49 ± 0.07; intermediate phase 5.98 ± 0.67[a] | 42.66 ± 2.04 | 5.46 ± 0.6 |
| Pepin et al.[51] | Horse F(ab′)2 | Rabbit | 209 ± 24 | 2.66 ± 0.18 | 49.69 ± 4.13 | 3.33 ± 0.38 |
| Pepin et al.[51] | Horse F(ab′)2 | Rabbit | 265 ± 34 | 2.66 ± 0.74 | 46.93 ± 5.92 | 3.96 ± 0.75 |
| Ismail & Abd-Elsalam[35] | Horse F(ab′)2 | Rabbit | | Rapid initial phase 0.2 ± 0.05; intermediate phase 0.95 ± 0.12[a] | 28.38 ± 3.24 | |
| Pepin-Covatta et al.[39] | Horse F(ab′)2 | Rabbit | 230 ± 26 | 2.54 ± 0.36 | 49.52 ± 3.07 | 3.56 ± 0.34 |
| Ismail et al.[36] | Horse F(ab′)2 | Rabbit | 216 ± 9 | Rapid initial phase 0.22 ± 0.05; intermediate phase 2.33 ± 0.45[a] | 33.84 ± 0.93 | 5.4 ± 0.54 |
| Rivière et al.[38] | Horse F(ab′)2 | Rabbit | 130 ± 5 | 2.9 ± 0.7 | 55 ± 9 | 2.1 ± 0.01 |
| Rivière et al.[38] | Horse Fab | Rabbit | 230 ± 2 | 0.7 ± 0.02 | 8 ± 0.3 | 53 ± 0.5 |

a Triphasic curves were obtained, with a rapid initial declining phase, followed by a slower intermediate phase and then the slowest terminal phase.

**CL** = clearance; $t_{½\alpha}$ = distribution half-life; $t_{½\beta}$ = elimination half-life; **Vd** = volume of distribution.

elimination half-life when compared with IgG and F(ab′)2,[40,42,44,45] in agreement with the high clearance of digoxin-specific Fab molecules.[43,46,47]

In a study of patients envenomated by the African viperid snake *Echis ocellatus*, an ovine Fab antivenom was cleared 4–5 times faster than a horse F(ab′)2 antivenom.[44] Antivenoms made of F(ab′)2 fragments remain in the circulation for longer periods of time than antivenoms made of Fab fragments.[40,41,44,45] The terminal half-life of equine or ovine antibodies administered in humans is shorter than that of homologous human IgG preparations, suggesting accelerated clearance mechanisms that enhance the removal of heterologous immunoglobulins and their fragments in humans.[50] However, the nature of these mechanisms has not been fully elucidated.

## 1.3 Experimental Studies

### *1.3.1 Absorption and Distribution After Intravenous Administration*

A number of studies have been performed on antivenom pharmacokinetics in animal models, mainly rabbits, although rats and mice have also been used. Most authors describe, for snake and scorpion antivenoms, a biexponential decline in the plasma levels of IgG, F(ab′)2 or Fab, compatible with a two-compartment model.[38,39,51] However, other studies describe a triexponential decline which fits a three-compartment open pharmacokinetic model including a central compartment (blood), a rapidly equilibrating 'shallow' tissue compartment and a slowly equilibrating 'deep' tissue compartment.[35,36,52] In general terms, the molecular mass of antibodies and antibody fragments greatly determines their pharmacokinetic parameters (table II). Fab and F(ab′)2 generally have larger volumes of distribution than IgG, implying that the former two diffuse to the extravascular compartment to a greater extent than IgG (table II). These differences have potential pharmacodynamic implications, since they determine the likelihood that an antibody, or an antibody fragment, may complex with a toxin in the tissue compartment.

Fab distributes more rapidly to the tissues than do F(ab′)2 and IgG.[35,38,52] Moreover, the elimination half-life of Fab is shorter than that of F(ab′)2 and IgG,[35,38,39,51] a feature clearly associated with the significantly higher clearance of Fab.[38,39,51] Fab

fragments are cleared from the body 35 times faster than IgG,[53] mainly due to renal elimination associated with its low molecular mass. IgG and F(ab′)2 remain in the circulation for a longer period of time.[53] The pharmacokinetic characteristics of F(ab′)2 lie between those of IgG and Fab.[53] The lower the molecular mass of antibodies and their fragments, the higher their diffusion between capillary plasma and interstitial fluid in the various organs.[53] The liver is an exception, since it has a higher uptake of IgG, showing that factors other than molecular size play a role in the extravasation of IgG in this organ.[53] It has been proposed that receptor-mediated uptake, probably dependent on recognition of the Fc region of the IgG molecule, is involved in this phenomenon.[53]

The net number of cycles per gram of tissue, through the interstitial and cell-associated regions, is an important pharmacokinetic parameter, since it reflects the extent of exposure of antibodies, or their fragments, to the milieu where venom components may be located in a tissue. In this regard, IgG cycles 17 times and 35 times more often than F(ab′)2 and Fab, respectively, in most organs.[53] Of particular relevance is the observation that IgG has greater chance than F(ab′)2 and Fab fragments of cycling the interstitial space in the carcass, which includes skeletal muscle.[53] Since snake venom myotoxins and α-neurotoxins, as well as other animal toxins, act at the skeletal muscle plasma membrane,[7,54] this observation may have pharmacodynamic implications. Thus, Fab fragments have larger volume of distribution and diffuse more rapidly than IgG, reaching higher interstitial fluid : plasma concentration ratios. However, Fab fragments are cleared from the body much faster than F(ab′)2 and IgG and, with the exception of kidneys, cycle through interstitial spaces less often than the latter.[53] The distribution of IgG and its fragments in the extravascular compartment is directly related to the permeability of capillaries of the various tissues. The liver, spleen and kidneys, having the highest plasma flow and permeability-surface area, also show the highest values for cycling of IgG, F(ab′)2 and Fab through the interstitial space.[53]

#### *1.3.2 Absorption and Distribution After Intramuscular Administration*

The pharmacokinetics of antivenoms after intramuscular administration has been investigated, since this route is sometimes employed when antivenoms are used in the field. As depicted in table III, using the rabbit as model, absorption of IgG and F(ab′)2 is slow, with time to peak concentration ($t_{max}$) ranging from 48–76 hours. In contrast, Fab fragments are absorbed more readily, with a $t_{max}$ of 12 hours.[55] Bioavailability of F(ab′)2 fragments by this route is 36–42%, and elimination half-life is longer for F(ab′)2 and IgG antivenoms than for Fab antivenoms (table III), in agreement with observations carried out after intravenous injection. Thus, antibodies and their fragments reach the bloodstream at a relatively slow rate after intramuscular administration, and a significant proportion of antibody molecules do not reach the circulation at all, thereby decreasing the effectiveness of this route of administration in the treatment of envenomations.

**Table III.** Pharmacokinetic parameters of antivenoms after intramuscular administration in experimental studies. Values are mean ± SD or mean ± SEM

| Study | Type of antivenom | Animal model | Bioavailability (%) | $t_{max}$ (h) | $t_{½\beta}$ (h) |
|---|---|---|---|---|---|
| Ismail & Abd-Elsalam[35] | Horse IgG | Rabbit | | 76.3 ± 5.3 | |
| Ismail & Abd-Elsalam[35] | Horse F(ab′)2 | Rabbit | | 48 ± 2.5 | |
| Pepin et al.[51] | Horse F(ab′)2 | Rabbit | 42 | 48 | 59.63 ± 2.88 |
| Pepin-Covatta et al.[39] | Horse F(ab′)2 | Rabbit | 36 | 48 | 57.80 ± 1.80 |
| Rivière et al.[55] | Horse Fab | Rabbit | | 12 | 13.2 ± 0.3 |

$t_{max}$ = time to reach maximum concentration in plasma; $t_{½\beta}$ = elimination half-life.

#### 1.3.3 Elimination

Fab fragments are readily filtered in the glomerulus, being reabsorbed and catabolised by proximal tubular cells.[56] In contrast, the larger IgG and F(ab′)2 molecules cannot be filtered at the glomerular level, thus remaining in the circulation for longer periods of time. The renal handling of Fab may have effects on renal function which, in turn, would affect Fab elimination. Experimental observations showed a reduction in creatinine clearance after administration of digoxin-specific Fab fragment to rabbits,[57] suggesting that Fab affects the glomerular filtration rate in this model, whereas tubular function was not affected. Coincidentally, administration of Fab antivenom in rabbits injected with *Vipera aspis* snake venom was associated with a reduction in the volume of urine, a phenomenon not observed when rabbits were treated with an F(ab′)2 antivenom.[55] Thus, although no renal alterations have been reported in any of the clinical studies carried out with ovine Fab antivenoms,[44,45,58] careful attention needs to be given to this possibility in future clinical trials, especially when dealing with envenomations that impair renal function, such as those inflicted by some viperid, hydrophid and elapid snakes.[1,59]

In mice, most of the catabolism of IgG takes place in gut and liver, whereas gut and kidney predominate in the case of F(ab′)2, the kidney being predominant for Fab fragments.[53] In the case of IgG molecules with intact Fc portions, which contain carbohydrate moieties, carbohydrate-specific uptake mechanisms associated with the mononuclear phagocyte system in liver and other organs play a role in their catabolism.[50,53] Aggregated IgG molecules are removed by hepatic cells more readily than is monomeric IgG,[60,61] showing that the degree of oligomerisation directly affects the uptake of antibodies by the liver. Since antivenoms usually contain molecular aggregates of immunoglobulins or their fragments,[33] the extent of molecular aggregation in a particular product may affect its rate of uptake and catabolism by liver cells. In an attempt to identify the sites of IgG catabolism in rats, it was observed that, on a weight basis, liver and spleen have the highest activity, particularly in hepatic nonparenchymal cells.[62] However, in terms of total catabolism, peripheral tissues, skin and muscle, play the dominant role, indicating that the catabolism of antibodies seems to take place diffusely throughout the body.[62] Nevertheless, it is necessary to assess the elimination pathways of equine and ovine IgG, F(ab′)2 and Fab, since significant differences may exist depending on the origin of the antibody and on the animal model used.

#### 1.3.4 Effects of Envenomation on Antivenom Pharmacokinetics

Few studies have addressed the subject of pharmacokinetics of antivenoms in envenomated animals, since the vast majority of studies have been performed in normal animals, as discussed above. The local vascular alterations, i.e. haemorrhage and oedema, associated with local tissue damage in viperid snake envenomations[13,63,64] drastically affect the permeability of microvessels in those sites. This has obvious implications for the distribution and extravasation of antibodies and their fragments. Experimental observations carried out in mice showed that both IgG and F(ab′)2 accumulate to a significantly higher extent in envenomated gastrocnemius muscle than in control muscle.[65] A similar increment in antibody extravasation in muscle was observed with the venom of the elapid snake *Micrurus nigrocinctus*, which induces oedema but not haemorrhage, reflecting that less drastic alterations in the microvasculature also promote extravasation.[66] Interestingly, in the case of mice envenomated with *Bothrops asper* venom, this enhanced accumulation in muscle tissue occurred to a similar extent for IgG and F(ab′)2, indicating that the microvascular alterations facilitate a similar extravasation of both types of molecules, irrespective of their different molecular masses.[65]

Viperid snake venoms induce a systemic increase in vascular permeability, as well as systemic bleeding, associated with widespread extravasation and hypovolaemia.[1,59] Therefore, it is likely that pharmacokinetic parameters such as volume of distribution, time to reach maximum tissue concentration and number of cycles that antibodies have in each tissue vary in envenomated animals when compared with control non-envenomated animals. Levels of horse IgG, after intravenous administration of antivenom, were lower in mice previously injected intramuscularly with *B. asper* venom than in control mice.[67] Although this may be due to venom sequestration after antibody binding, it may also reflect an enhanced extravasation of IgG in these animals. The study of pharmacokinetics of antivenoms in envenomated animals is a rather neglected subject that deserves careful consideration.

The kinetics and routes of elimination of IgG and its fragments also vary after binding to venom antigens, reflecting an additional difference in antibody pharmacokinetics between normal and envenomated individuals. For Fab, such variation is greatly influenced by the molecular mass of the toxins bound. In the case of viperid snake venoms, in which HMM proteins are abundant, the Fab-venom complexes are eliminated at a slower rate than free Fab fragments, since such complexes are not eliminated by the renal route, thus remaining in the circulation for a longer time.[49,55] This may not happen when Fab fragments bind to LMM toxins, such as scorpion and elapid and hydrophid snake neurotoxins. In these cases, Fab-toxin complexes might still be predominantly eliminated by the renal route, as described in the case of the small molecules colchicine and digoxin.[50] On the other hand, the elimination of complexes formed between IgG and $F(ab')_2$ and toxins probably takes place by phagocytosis at the mononuclear phagocyte system throughout the body.[55]

## 2. Pharmacodynamics

### 2.1 Neutralisation of Toxins by Antibodies and Their Fragments

The molecular basis of toxin neutralisation by antibodies or their fragments is the blockade of the molecular regions of the toxins involved in the interaction with their targets, precluding their pharmacological action. Such blockade may occur by various direct or indirect mechanisms. (i) The 'pharmacological site' of the toxin may be an epitope recognised by antibody paratopes. (ii) Antibodies may bind to epitopes located in the vicinity of the 'pharmacological site' of the toxin, neutralisation being achieved by steric hindrance of such a molecular region, with the consequent inability to reach its target. (iii) Antibodies may recognise an epitope far from the 'pharmacological site' of toxins, inducing conformational changes in the molecule that decrease its affinity for cellular targets. This mechanism would be involved in the described antibody-induced reversion of the binding of toxins to receptors.[22] (iv) IgG molecules or $F(ab')_2$ fragments may form multivalent immunocomplexes with toxins, and such complexes may be removed by phagocytic cells, eliminating the toxin from relevant tissue locations. This mechanism does not operate in the case of Fab fragments since, being monovalent, they are unable to form multivalent linkages with antigens. Antibody binding to toxins may occur in plasma or in interstitial fluid, or may take place after the toxin binds to its cellular or extracellular target. Neutralisation can occur in both circumstances, as it has been demonstrated that antibodies are able to reverse the binding of snake venom α-neurotoxins already bound to the motor end-plate cholinergic receptor.[68-70]

The affinity and avidity with which antibodies and their fragments bind to toxins greatly determine their potential therapeutic success.[50] Antibodies of

high affinity are less likely to dissociate from antigen-antibody complexes and a have a higher possibility of removing toxins already bound to their targets. Moreover, such antibodies would be more successful at promoting redistribution of toxins from tissue compartments to blood (see section 2.4). By using biosensor technology, the affinity constants of two $F(ab')_2$ antivenoms for *V. aspis* snake venom were found to be approximately $10^8$ L/mol.[71] However, the routine quality control of the efficacy of antivenoms does not involve the direct assessment of antibody affinity. Instead, it is based on neutralisation assays in which venom and antivenom are mixed and incubated before standardised testing in laboratory animals.[72-76] Most manufacturers assess only the ability of antivenoms to neutralise the lethal effect of a particular venom, although it has been proposed that antivenoms should be assayed also for their ability to neutralise other pharmacologically relevant effects of venoms.[72,74,77] In the case of viperid snake venoms, the neutralisation of myotoxic, necrotising, haemorrhagic, oedema-forming, coagulant and defibrinating effects needs to be tested in order to have an integral assessment of their preclinical neutralising efficacy.[72,74,77]

The effectiveness of immunotherapy in envenomations depends on the ability of antibodies, or their fragments, to bind, extract or redistribute, and eliminate toxins present in various locations in the body.[50] With the exception of endocytosed toxins having an intracellular site of action, venom components may be either bound to a membrane target, e.g. neurotoxins affecting ion channels, located in the extracellular matrix, e.g. snake venom metalloproteinases, or circulating in the bloodstream, e.g. procoagulant enzymes present in snake venoms. Thus, depending on the nature and site of action of a given toxin, as well as on the time of onset of its action, antibodies and their fragments would have different probabilities of binding and neutralising these toxins. Therefore, the pharmacokinetics of venoms and antivenoms play a critical role in the success of neutralisation, since toxin sequestration by antibodies has to occur in a common distribution space and, ideally, before the toxins exert their deleterious effects.

### 2.2 Venom Pharmacokinetics Differ from Antivenom Pharmacokinetics

The probability of interaction of antibodies or their fragments with toxins depends on their volumes of distribution. A notorious mismatch has been described between venoms and antivenoms in regard to this pharmacokinetic parameter, as well as in the time needed to reach equilibrium in the distribution space. In the case of scorpion venom neurotoxins, their low molecular mass favours a rapid distribution in the extravascular compartments, with much shorter distribution and elimination half-lives than those of IgG, $F(ab')_2$ and Fab.[35,52,78] In addition, the time needed by the venoms of the scorpions *Androctonus amoreuxi, A. crassicauda, Leiurus quinquestriatus, Buthotus judaicus, Buthus occitanus, Tityus serrulatus* and *Centruroides limpidus* to reach maximum tissue concentrations is much shorter than that of IgG and its fragments.[35,39,52,78-80] This observation is compatible with the rapid appearance of symptomatology in patients stung by scorpions.[5] Moreover, scorpion toxins have a higher affinity for tissues than do IgG and its fragments, as shown by the higher intercompartmental transfer rate constants from the vascular compartment.[35,79]

A similar pharmacokinetic mismatch occurs between antibodies or their fragments and the LMM neurotoxins of the elapid snake *Walterinnesia aegyptia*. Rapid absorption and distribution of crude venom and purified neurotoxin in the tissue compartment were described, with much shorter distribution and elimination half-lives than those of horse IgG, $F(ab')_2$ and Fab.[36]

An additional pharmacokinetic discrepancy between LMM neurotoxins and antibodies has to do with their bioavailability and time to reach maximum concentrations in blood after intramuscular or subcutaneous injection. Bioavailability of $F(ab')_2$ in rabbits is 36–42%,[39,51] and $t_{max}$ is 12 hours for Fab,[55] 48 hours for $F(ab')_2$[35,39,51] and 76 hours for IgG.[35] In contrast, approximately 90% of the total injected dose of *W. aegyptia* venom was absorbed within 60 minutes,[36] and $t_{max}$ for scorpion venoms injected intramuscularly or subcutaneously was less than 2 hours.[36,39,52,79-82] Rapid absorption was also described in rabbits and mice injected subcutaneously with venom of the elapid snake *M. nigrocinctus*.[83] A rapid distribution of 'crotoxin', a heterodimeric neurotoxic phospholipase $A_2$ from the venom of the South American rattlesnake *Crotalus durissus terrificus*, was also described in human patients[84] and after experimental subcutaneous injection in mice.[85]

A different pharmacokinetic pattern was described for viperid snake venoms, in which HMM proteins are abundant. Despite the presence of phospholipase $A_2$ (monomeric molecular mass 14 kDa) and other small proteins and polypeptides, viperid venoms contain abundant metalloproteinases and serine proteinases of 20–100 kDa.[12,86] These proteinases are responsible for haemorrhage and coagulopathies, which are the predominant systemic alterations described in viperid snake envenomations.[1,59,87,88] In the case of *V. aspis* venom, the pharmacokinetic parameters of the HMM fraction differ from those of the LMM proteins, since the former show a smaller volume of distribution and a higher elimination half-life than the latter.[89] The volume of distribution of the HMM fraction was larger than blood volume, suggesting distribution to the tissue compartment.[89] When *V. aspis* venom was injected intramuscularly, as often occurs in actual snakebites, there was a complex kinetics of venom absorption. An initial phase of rapid absorption was followed by a slow absorption which persisted for 72 hours after injection.[89] Similar observations were carried out with the venom of the South American viperid *Bothrops jararaca*.[90] Such prolonged venom absorption from the injection site has been associated with a phenomenon of recurrence of envenomation described in clinical studies (see section 2.6). Venom pharmacokinetics may be altered by first aid measures commonly used in many countries. For instance, application of tourniquets has been reported to delay venom absorption and the onset of clinical signs in snakebitten patients.[91] The pharmacodynamic implications of the described extensive mismatch in the pharmacokinetics of venoms and antivenoms is a key issue in the immunotherapy of envenomations.

## 2.3 Comparison of Venom Neutralisation by Antibody and Antibody Fragment Antivenoms

### *2.3.1 Experimental Studies on Neutralisation of Systemic Effects*

When choosing the most appropriate type of antivenom [IgG, $F(ab')_2$ or Fab], it is desirable that the ratio of the volume of distribution of the toxin to that of the antibody fragment approaches unity.[50] On theoretical grounds, toxins with large volumes of distribution and rapid absorption from the injection site, such as snake and scorpion venom neurotoxins, would be better neutralised by Fab and $F(ab')_2$ fragments, which have larger volumes of distribution than IgG molecules. The successful use of Fab fragments in the treatment of digitalis toxicity,[92] as well as in cardiotoxicity due to ingestion of yellow oleander seeds,[93] supports the idea that Fab antivenoms are likely to be effective in binding and neutralising toxins that have extensive distribution in the tissues.

This hypothesis has been tested in 'rescue' experiments, in which a lethal dose of a neurotoxic venom is injected in experimental animals, and antivenoms are administered intravenously afterwards, at vari-

ous time intervals. When venom of the elapid snake *W. aegyptia* was injected in rats, a complete neutralisation of lethality was achieved with F(ab')2 antivenom, if administered rapidly. Neutralisation by IgG antivenom was only partial, whereas no neutralisation was observed with Fab fragments.[36,52] In a similar study performed in mice injected with the venom of the elapid snake *M. nigrocinctus*, no difference was found between IgG and F(ab')2 antivenoms in the neutralisation of lethality.[66] However, the time of death of animals receiving a subneutralising dose of F(ab')2 immediately after envenomation was more prolonged than that of mice treated with IgG.[66]

In another investigation, both IgG and F(ab')2 antivenoms were effective in preventing death of rats injected with a lethal dose of the venom of the scorpion *L. quinquestriatus*, when administered intravenously 20–30 minutes after envenomation.[35,52] In contrast, an Fab antivenom was only partially effective.[52] If immunotherapy was delayed for 40 minutes, F(ab')2 antivenom had the highest efficacy in 'rescue' experiments, whereas IgG antivenom was less effective, and Fab antivenom showed the lowest efficacy.[52] When studying the influence of pharmacokinetics on the pharmacodynamics of antivenoms, it is recommended to compare antivenoms having a similar neutralising efficacy in assays involving preincubation of venom and antivenom. In these circumstances, the differences observed in 'rescue' assays would mainly depend on the different pharmacokinetic characteristics of the neutralising molecules.[94,95]

These experimental observations indicate that although the volume of distribution of IgG, F(ab')2 and Fab may influence the effectiveness of antivenoms, there does not seem to be a strict correlation between volume of distribution and efficacy, thus suggesting that other pharmacokinetic factors are involved. One of these factors is likely to be the net number of cycles of IgG, F(ab')2 and Fab through the interstitial space in each organ. IgG shows the greatest number of cycles, followed by F(ab')2 and Fab.[53] Thus, in the case of Fab, its large volume of distribution and its short equilibration time allows it to rapidly reach tissue sites where LMM toxins might be present. Nevertheless, its low number of cycles in each organ, associated with a rapid clearance, reduce its possibilities of interacting with such toxins, affecting the neutralisation of toxins at later stages in the course of envenomation. In contrast, IgG molecules have a lower volume of distribution and diffuse to the extravascular compartment at a slower rate, precluding a rapid binding to venom components present in the interstitial space. However, its high number of cycles per organ increases the probability of toxin neutralisation at later time intervals.

'Rescue' experiments have also been performed in mice with the venom of the viperid snake *B. asper*, in which lethality is due to HMM proteins (our unpublished observations). No differences were found in the neutralising performance of IgG and F(ab')2 antivenoms.[65] Similar results were also obtained with South American viperid snake venoms.[96] In addition, these antivenoms had similar effectiveness in the neutralisation of defibrinating effect induced by the venoms of *B. asper*[65] and *B. jararaca*.[97] These observations might be due to the fact that lethal and defibrinating components of these venoms must reach the bloodstream to exert their effects. Since antivenoms were administered intravenously, and since IgG and F(ab')2 remain in the circulation for relatively long periods of time, it is likely that toxins are bound and neutralised to a similar extent by circulating IgG and F(ab')2 once they reach the bloodstream.[65] Most studies comparing the efficacy of antivenoms when administered after envenomation have been performed with IgG and F(ab')2 antivenoms. Comparative investigations with Fab antivenoms are needed to have a deeper understanding of this complex issue.

The selection of the most adequate type of neutralising molecule in the immunotherapy of envenomation depends on the characteristics of the toxin being neutralised. If a particular toxin diffuses and acts rapidly in targets located in the tissue compartment, it would be beneficial to have an antibody fragment with rapid access to the tissue. If this is the case, Fab fragments probably have advantages over IgG and F(ab′)2 on theoretical grounds, since a rapid binding of toxins may be more relevant for neutralisation than a repeated cycling through the tissue. Nevertheless, this hypothesis has not been supported by experimental nor clinical studies, and needs to be further tested in various venom-antivenom systems. The rapid clearance of Fab fragments might hamper their therapeutic potential, a phenomenon that would also occur with the even smaller Fv fragments.[50] This hypothesis has yet to be tested. On the other hand, the clinical success of F(ab′)2 antivenoms in the treatment of scorpion envenomations[5,98,99] is clear evidence that F(ab′)2 fragments are able to neutralise small neurotoxins after envenomation.

In the case of HMM toxins that may reach the tissues at a slower rate and act in a more delayed fashion, a neutralising molecule with the ability to cycle many times through interstitial space, and with a longer elimination half-life, may be more suitable for neutralisation. Moreover, when dealing with toxins that exert their actions in the bloodstream, for example snake venom procoagulant enzymes, the presence of a sustained, high concentration of antibodies or their fragments in blood is required to assure neutralisation. On the basis of their pharmacokinetic characteristics, this would be achieved better by F(ab′)2 and IgG antivenoms than by Fab antivenoms. The maintenance of high concentrations of neutralising molecules in blood also promotes the redistribution of toxins from tissue locations (see section 2.4). The complexity and variety of the different venom-antivenom systems preclude simplistic generalisations and urge detailed experimental and clinical assessments of individual types of envenomations, in order to select the most appropriate type of neutralising molecule.

#### 2.3.2 Experimental Studies on Neutralisation of Local Tissue Damage

Some spider venoms, as well as many snake venoms, particularly those of viperid species, induce prominent local tissue damage.[4,14] In the case of viperid snake venoms, this damage is characterised by oedema, haemorrhage, myonecrosis and dermonecrosis.[1,54,59,63,87,100] Tissue damage usually occurs rapidly after venom injection, presenting problems for successful neutralisation by antivenoms, particularly when immunotherapy is performed late in the course of envenomation.[14,101] A complete abrogation of *B. asper* venom-induced local effects is not achieved experimentally even when mice are pretreated with antivenom before envenomation.[102]

It has been suggested that Fab and F(ab′)2 antivenoms, having higher volumes of distribution than IgG antivenoms, and reaching extravascular compartments more rapidly than the latter, would be more effective in the neutralisation of venom-induced local effects. However, experimental studies in mice do not support this hypothesis, as IgG, F(ab′)2 and Fab antivenoms neutralise local effects induced by *B. asper* and *Vipera berus* venoms in a quantitatively similar way (figure 1).[94,95,103] In the case of myotoxic effect induced by *B. asper* venom, an IgG antivenom showed higher efficacy than a Fab antivenom.[95] Moreover, in agreement with the rapid development of *B. asper* venom-induced effects, neutralisation is achieved only to a partial extent, even when antibodies and their fragments are administered intravenously immediately after envenomation (figure 1).[94,95] Similar observations were performed regarding neutralisation of local myotoxicity induced by *M. nigrocinctus* coral snake venom.[66]

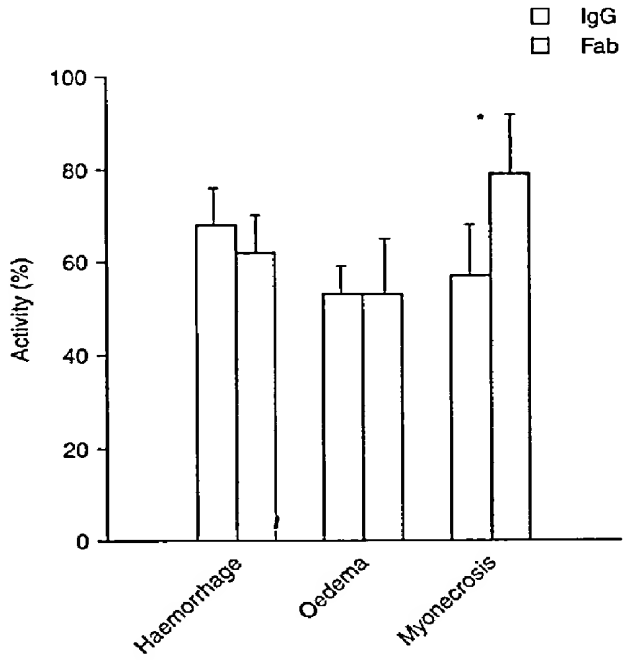


**Fig. 1.** Neutralisation of local effects (haemorrhage, oedema and myonecrosis) induced by the venom of the snake *Bothrops asper* in mice. Animals received an intramuscular injection of venom in the right gastrocnemius muscle. Immediately after envenomation, animals received an intravenous administration of either IgG antivenom or Fab antivenom. Control mice were injected with venom alone and then with saline solution instead of antivenom. The extent of each pathological effect was assessed by standard laboratory assays and expressed in percentage, 100% corresponding to the effect induced by venom in control mice treated with saline solution instead of antivenom. Notice that neutralisation of the three toxic effects was achieved only to a partial extent. Moreover, no significant differences were observed between antivenoms regarding their capacity to neutralise haemorrhage and oedema, whereas IgG antivenom was more effective than Fab antivenom in the neutralisation of myonecrosis. * indicates statistical significance, $p < 0.05$. See León et al.[95] for details.

The similar and incomplete efficacy of IgG, F(ab′)2 and Fab antivenoms to neutralise locally-acting toxins, despite their markedly different pharmacokinetic profiles, may be explained on two grounds. First, tissue damage occurs so rapidly that antibodies and their fragments reach interstitial fluid at a time when significant pathological effects have occurred.[64,104,105] In this context, the pharmacokinetic differences between antivenoms would be of little relevance to the extent of neutralisation. Secondly, the effects induced by viper snake venoms in the microvasculature at the site of injection, i.e. increase in vascular permeability and haemorrhage,[63,64,105] would change the pharmacokinetics of antivenoms, favouring a pronounced extravasation of IgG, F(ab′)2 and Fab, irrespective of their molecular masses. This was corroborated in the case of IgG and F(ab′)2, since a drastic increment in the accumulation of these molecules was found in envenomated muscle when compared with control muscle.[65] Despite their significant variation in size, there was a similar increment in IgG and F(ab′)2 levels in muscle.[65] This partially explains the similar extent of neutralisation of locally acting toxins by these antivenoms.

In agreement with what has been observed in clinical studies,[14,58,87] these experimental observations emphasise the difficulty in neutralising locally acting toxins present in snake venoms. When dealing with *B. asper* snake venom, the local application of a polyvalent IgG antivenom, at the site of envenomation, does not improve neutralisation,[106] probably due to poor diffusion of antibodies. However, in the case of local experimental envenomation by the spider *Loxosceles deserta*, which induces a drastic necrotising effect in a rabbit model, local intradermal application of Fab fragments was effective in reducing local necrosis and neutrophil accumulation, when administered early in the course of envenomation.[107] Since local necrosis induced by this spider venom has a slower onset than snake venom-induced tissue damage, this time span probably allows Fab fragments to reach the toxins before damage has occurred. Nevertheless, no comparison was performed with IgG and F(ab′)2 antivenoms.

In conclusion, immunotherapy of venom-induced local tissue damage represents a difficult challenge. It has been proposed that local injection of potent enzyme inhibitors of high diffusibility may be an alternative to circumvent the problem associated with the poor efficacy of antivenoms when treating viperid envenomations.[108] This hypothesis has been supported at the experimental level with

the peptidomimetic hydroxamate metalloproteinase inhibitor batimastat (BB-94), which has proven to be effective at counteracting metalloproteinases from *B. asper* snake venom if injected rapidly after envenomation.[109,110] Local application of natural or synthetic enzyme inhibitors may represent a future therapeutic tool to complement immunotherapy in some envenomations.

#### 2.3.3 Experimental Studies Involving Intramuscular Administration of Antivenoms

In the clinic, antivenoms are generally administered by intravenous infusion after dilution with saline solution.[59] However, when immunotherapy is performed in the field, antivenoms are often administered intramuscularly.[59] In agreement with the described pharmacokinetic profile of intramuscular injected immunoglobulins and fragments, this route is far less effective than the intravenous route. Bioavailability of $F(ab')_2$ fragments by intramuscular injection is ~40%,[39,51] and $t_{max}$ ranges from 12 hours for Fab to 76 hours for IgG.[35,55] These values contrast with the rapidity by which toxins reach the bloodstream and distribute to their tissue targets. Accordingly, experimental neutralisation studies show a poor efficacy of antivenoms, when given intramuscularly, in preventing death after injections of lethal doses of scorpion venoms and neurotoxic snake venoms.[35,111] Moreover, antivenoms administered intramuscularly are also ineffective in neutralising haemorrhagic and muscle-damaging toxins from the venom of the snake *B. asper*.[106] Recent observations indicate that an Fab antivenom is also ineffective, when administered intramuscularly in a mouse experimental model, in the neutralisation of lethality, myotoxicity and haemorrhage induced by *B. asper* venom (our unpublished results). Thus, pharmacokinetic and pharmacodynamic observations clearly agree in that the intramuscular route for immunotherapy of envenomations has poor efficacy and that antivenoms should preferentially be administered intravenously.

#### 2.3.4 Clinical Investigations Comparing Antivenoms

In contrast with the large amount of information available on the efficacy of individual antivenoms in treating snake or scorpion envenomations, there are relatively few randomised clinical trials comparing the efficacy of antivenoms made of IgG, $F(ab')_2$ or Fab molecules. One study compared a goat IgG antivenom with an unrefined equine serum antivenom and a $F(ab')_2$ antivenom, all monospecific against *Calloselasma rhodostoma* snake venom.[40,112] IgG and $F(ab')_2$ antivenoms produced rapid and permanent restoration of blood coagulability, the latter showing the highest increase in plasma fibrinogen concentration within 24 hours. Unrefined antivenom was less efficient in restoring blood coagulability.[112] There were no differences between treatments in the neutralisation of local swelling nor in the changes in various haematological parameters.[112]

Two randomised trials compared Fab and $F(ab')_2$ antivenoms for the treatment of envenomations by the snakes *Echis ocellatus* and *Daboia russelli russelli*.[44,45] Fab and $F(ab')_2$ antivenoms were equally effective in clearing *E. ocellatus* venom antigenaemia, and two-thirds of patients from both groups required further doses of antivenoms because coagulopathies did not resolve within 6 hours, or recurred. The authors concluded that a larger initial dose, or repeated doses, of these antivenoms are needed for complete neutralisation of the venom.[44] In the other study, an $F(ab')_2$ antivenom was more effective than an Fab antivenom in restoring blood coagulability and in stopping the progress of swelling and other clinical manifestations in patients bitten by *D. r. russelli*.[45] A larger initial dose of Fab antivenom was recommended for future trials. In addition, one $F(ab')_2$ and two IgG antivenoms were compared for the treatment of envenomations by *Bothrops* sp. snakes in Colombia.[113] The three antivenoms were effective, and no significant differences were found regarding time of cessation of

bleeding, halting the progression of local swelling and restoration of blood coagulability.

### 2.4 Antibody-Induced Venom Redistribution

Both experimental and clinical investigations have demonstrated a rapid immunocomplex formation of antibodies or their fragments with venom components in the circulation, which depends on the affinity between antibodies and toxins. The higher the affinity of antibodies to toxins, the lower the probability of dissociation of the complexes. When effective antivenoms are used, there is a drastic drop in plasma free venom concentration after intravenous infusion of antivenom, a phenomenon extensively documented in clinical studies.[41,45,87,88,91,98] This is a clinically relevant finding, since a definite correlation has been observed between venom levels in blood and the severity of envenomation.[41,87,88,114-116] In contrast, ineffective antivenoms fail to completely eliminate free venom in plasma, and allow late increments in plasma venom levels, both of which are linked with recurrence of clinical signs.[91,117,118]

Complex formation between antibodies and toxins in the circulation is associated with a redistribution of venom toxins from the tissue compartment to the vascular compartment,[38,39,71,80] although it has been suggested that such venom redistribution occurs from the lymphatic circulation to blood.[55] Venom redistribution was shown, in experimental studies with scorpion venoms, by an increment in total venom concentration in plasma after immunotherapy.[39,80] A similar phenomenon was described for *V. aspis* snake venom after infusion of an $F(ab')_2$ antivenom.[38,71] Immunotherapy was followed by a sharp drop in free venom levels in plasma, together with an increase in total venom concentration.[38] Such modification of venom pharmacokinetics depends not only on the affinity of antibodies for toxins, but also on the dose of antivenom and the delay in antivenom administration.[38,71] In contrast to what was described for $F(ab')_2$ antivenom, redistribution and immunoneutralisation of *V. aspis* venom were not complete when a Fab antivenom was used, since relatively high levels of free venom were detected in plasma after antivenom infusion.[38] This observation is probably explained by the pharmacokinetic profile of Fab fragments, as their large volume of distribution leads to a reduction in plasma levels. Thus, in promoting complex formation with venom proteins in blood, and redistribution of venom from tissue to the central compartment, $F(ab')_2$ and IgG antivenoms seem to be more effective than Fab antivenoms.

The described redistribution effect may involve the removal of toxins from their tissue targets. The likelihood of displacement of toxins from their targets and their redistribution to the central compartment is directly proportional to the affinity between antibodies and toxins, and inversely proportional to the affinity of toxins for their targets.[50] In the case of toxins that bind cellular targets but without causing irreversible effects, such removal results in the abrogation of the pharmacological effect induced by the toxin. This has been described for scorpion and snake neurotoxins that bind to ion-channels or cholinergic receptors but do not cause their permanent dysregulation.[69,119] Thus, a goal of immunotherapy is to neutralise circulating toxins and to remove the toxins from their targets before neurotoxic effects have become life-threatening. A different scenario occurs with toxins that cause tissue damage, such as myotoxic[54] and haemorrhagic[13] viperid venom components, or presynaptically acting snake venom phospholipase $A_2$ that damages the integrity of the nerve terminal.[120] In these cases, antibody-induced redistribution halts further tissue damage, but does not restore normal function of the affected target, possibly leading to irreversible damage.[95,101,106] This difference has clinical implications, since it emphasises the relevance of early administration of antivenoms in order to promote venom redistribu-

tion to the central compartment and neutralisation before extensive tissue damage occurs. Coincidentally, the clinical efficacy of antivenoms in preventing snake venom-induced local tissue damage and presynaptic neurotoxicity is not particularly convincing.[14,91]

### 2.5 Antibody-Induced Venom Elimination

The kinetics of venom elimination is affected after complex formation with antibodies. In the case of LMM toxins, such as scorpion and snake venom neurotoxins, they adopt the elimination properties of IgG, F(ab′)$_2$ or Fab.[39] In this regard, small toxins behave similarly to other small molecules like colchicine, since Fab-colchicine complexes are eliminated with the same terminal half-life as free Fab.[50] In the case of viperid snake venoms, which contain abundant HMM proteins, elimination of F(ab′)$_2$-venom complexes is slower than elimination of free venom, but faster than elimination of free F(ab′)$_2$.[55] In contrast, Fab-venom complexes are eliminated slower than free Fab,[55] since the molecular mass of the complex does not allow the glomerular filtration and renal elimination characteristic of free Fab. Relatively little is known about the routes of elimination of toxins bound to antibodies or their fragments, although in the case of complexes involving IgG and F(ab′)$_2$ it is likely that elimination is performed predominantly by phagocytosis in various organs.[55]

### 2.6 Recurrence of Envenomation and its Relationship to Antibody Fragment Pharmacokinetics

The reappearance of venom effects after an initial improvement following antivenom therapy was initially described in envenomations by the pit viper *Calloselasma rhodostoma*[112], and has been consistently observed in the clinical management of various envenomations, particularly after viperid snakebites.[40,42,44,58,112,121-123] This phenomenon has been associated with a delayed absorption of venom from the site of injection in the tissues,[58,112] although alternative mechanisms have been discussed.[58] This is another manifestation of the mismatch between the pharmacokinetics of venom toxins and antibodies or their fragments, as it is likely that such recurrence is associated with a drop in plasma levels of IgG, F(ab′)$_2$ or Fab, together with an increase in plasma free venom levels.[42,58,112]

In viperid snake envenomations, recurrence in venom antigenaemia is responsible for the reappearance of bleeding and coagulopathies.[40,44,58] Despite the fact that recurrence of envenomation has been described for IgG and F(ab′)$_2$ antivenoms,[112,113,123] it has been particularly frequent when using Fab antivenoms.[42,44,58,121] This is probably due to the short elimination half-life of Fab fragments, which results in reduced Fab levels in blood by the time late venom absorption from tissues occurs.[44,49] In the case of IgG and F(ab′)$_2$ antivenoms, their more prolonged elimination half-life facilitates the binding and neutralisation of venom components that reach the circulation later on.

This problem of recurrence when using an ovine Fab antivenom in the therapy of pit viper envenomations has been confronted in the US by adopting a schedule in which repeated antivenom doses are administered every 6 hours for 18 hours.[58,122] However, this approach increases the total volume of antivenom required per treatment, a particularly relevant issue in developing countries, where antivenoms are scarce and constitute a heavy economic burden for the patients or the public health systems.[124,125]

## 3. Conclusions

Antivenoms made of whole IgG molecules, divalent F(ab′)$_2$ fragments or monovalent Fab fragments represent the main therapeutic resource in envenomations induced by animal bites and stings. An ideal antivenom should be safe and effective. The discussion of factors affecting antivenom safety, despite

its relevance, is beyond the scope of this review. Regarding efficacy, antivenoms must fulfil several requirements. (i) High affinity towards the most relevant toxins of the venom being neutralised, to promote strong binding and neutralisation of their toxic activities. Although direct affinity determinations are not performed routinely, the fulfilment of this requirement involves the neutralisation of the most relevant toxic activities of venoms in preclinical studies. (ii) A pharmacokinetic profile that resembles that of the venom being neutralised. Thus, for venoms whose main toxic effects are induced by LMM toxins of rapid diffusion and large volume of distribution, an antivenom with a high volume of distribution and rapid equilibration in its distribution space would fill this requirement; and (iii) the maintenance of high antibody levels in plasma long enough to assure repeated cycling through the interstitial fluid of organs as well as neutralisation of venom components that may reach the circulation later on. In addition, elevated levels of high-affinity antibodies in blood favour the redistribution of venom components from tissues to blood. This requirement is particularly important for the neutralisation of venoms containing HMM toxins, such as those of many viperid snakes.

The complexity of venoms, many of which include both LMM and HMM toxins having different targets and mechanisms of action, makes the selection of the ideal pharmacokinetic profile of an antivenom a rather difficult and controversial task. Owing to this complexity, some antivenoms may have to include a mixture of Fab fragments and IgG or $F(ab')_2$ molecules. The former, having rapid equilibration and large volume of distribution, would allow rapid neutralisation of small toxins in tissues, whereas the latter would assure repeated cycling in tissues and high plasma levels for a relatively extended time. Nevertheless, the decision on the optimal characteristics of a particular antivenom must depend on a detailed analysis of the pharmacokinetics of the venom being neutralised, as well as on the understanding of the mechanisms and time-course of action of the toxins in that particular venom. Unfortunately, with very few exceptions, we are far from having a complete understanding of the pharmacokinetic-pharmacodynamic relationship of antivenoms, even for the neutralisation of the most relevant venoms from the medical viewpoint. Future efforts should focus on the enrichment of this knowledge and its application in the immunotherapy of envenomations.

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